static and suspension cultures by anti-human IgG1 ELISA and/or analytic Protein A HPLC. The selected clone was named I-17.

For the second transfection and selection step a plasmid with a hygromycin resistance gene has been used. The plasmid has been transfected with electroporation into cell line clone I-17 cultivated in ProCHO4-complete medium supplemented with 700 μg/ml G418. The transfected cells were expanded for about two to three weeks in ProCHO4-conditioned medium supplemented with 200 μg/ml G418 and 300 μg/ml hygromycin (ProCHO4-double selection medium). Single antibody secreting cells were identified and deposited on the basis of their fluorescence intensity after staining with a Protein A Alexa Fluor conjugate by FACS analysis. The deposited cells were cultivated in ProCHO4-double selection medium in 96 well plates. The antibody concentration in the culture supernatants was evaluated by an anti-human IgG1 ELISA. The selected clone was named 24_16. For the third transfection and selection step a plasmid with a puromycin resistance gene has been used. The plasmid has been transfected with electroporation into cell line clone 24_16 cultivated in ProCHO4-double selection medium. The transfected cells were expanded for about two to three weeks in ProCHO4-triple selection medium (ProCHO4-conditioned medium supplemented with 200 μg/ml G418 and 300 μg/ml hygromycin and 4 μg/ml puromycin). Single antibody secreting cells were identified and deposited on the basis of their fluorescence intensity after staining with a Protein A Alexa Fluor conjugate by FACS analysis. The deposited cells were cultivated in ProCHO4-triple selection medium in 96 well plates. The antibody concentration in the culture supernatants was evaluated by an anti-human IgG1 ELISA. The selected clone was named 1_24.

Clone Characteristics:

As can be seen from the following table the doubling time and the cell density after three days of cultivation were comparable when the basic cell line CHO-K1 (wild-type) and the selected clones are compared.

TABLE 5

Growth characteristics				
Clone	Doubling time [h]	Starting cell density [10^6 cells/ml]	Cell density at day 3 [10^6 cells/ml]	Viability at day 3 [%]
CHO-K1 (pre adapted)	22-25	3	18-22	96-98
I-17	25-30	3	13-15	95-97
24_16	25-30	3	15-16	95-96
1_24	30-32	3	12-14	95-97

The invention claimed is:

1. A method for the recombinant production of a heterologous immunoglobulin in a CHO cell which is secreted to the cultivation medium comprising:

a) providing a CHO cell, wherein said CHO cell is
adapted to growth in suspension culture,
adapted to growth in serum-free medium, and
mycoplasma free,

b) providing a nucleic acid comprising
i) a prokaryotic origin of replication,
ii) a first nucleic acid sequence conferring resistance to a prokaryotic selection agent,
iii) a second nucleic acid sequence encoding the heavy chain of said heterologous immunoglobulin, and a third nucleic acid sequence encoding the light chain of said heterologous immunoglobulin,
whereby a first transfection vector is provided which comprises said provided nucleic acid and an additional fourth nucleic acid sequence conferring resistance to a first eukaryotic selection agent,
whereby a second transfection vector is provided which comprises said provided nucleic acid and an additional fourth nucleic acid sequence conferring resistance to a second eukaryotic selection agent, whereby said second eukaryotic selection agent is different to said first eukaryotic selection agent,

b1) providing a nucleic acid comprising
i) a prokaryotic origin of replication,
ii) a first nucleic acid sequence conferring resistance to a prokaryotic selection agent,
iii) a second nucleic acid sequence encoding the heavy chain of said heterologous immunoglobulin, and/or a third nucleic acid sequence encoding the light chain of said heterologous immunoglobulin,
whereby a third transfection vector is provided which comprises said provided nucleic acid and an additional fourth nucleic acid sequence conferring resistance to a third eukaryotic selection agent, whereby said third eukaryotic selection agent is different to said first eukaryotic selection agent and is also different to said second eukaryotic selection agent,

c) transfecting said CHO cell, wherein said transfecting comprises the following:
(i) transfecting said CHO cell with said first transfection vector,
(ii) selecting a CHO cell transfected in (i) by selected growth in cultivation medium containing a first eukaryotic selection agent to which the first transfection vector confers resistance,
(iii) transfecting said selected CHO cell in (ii) with said second transfection vector,
(iv) selecting a CHO cell transfected in (iii) by selected growth in cultivation medium containing said first eukaryotic selection agent to which the first transfection vector confers resistance and said second eukaryotic selection agent to which the second transfection vector confers resistance,
(v) transfecting said CHO cell selected in (iv) with said third transfection vector,
(vi) selecting a CHO cell transfected in (v) by selected growth in a cultivation medium containing said first eukaryotic selection agent to which the first transfection vector confers resistance and said second eukaryotic selection agent to which the second transfection vector confers resistance and said third eukaryotic selection agent to which the third transfection vector confers resistance,

d) cultivating said transfected CHO cell in a medium in the presence of said first and said second and third eukaryotic selection agent, under conditions suitable for the expression of said second, and/or third nucleic acid, and

e) recovering said secreted heterologous immunoglobulin from the cultivation medium and thereby producing a heterologous immunoglobulin in a CHO cell which is secreted to the cultivation medium;

wherein said resultant CHO cell is stable in the absence of any or all selection agents, as used in the previous steps, for up to generation 60.

2. The method of claim **1**, wherein said CHO cell is selected from the group consisting of a CHO K1 cell, a CHO DG44 cell, a CHO XL99 cell, a CHO DXB11 cell, and a CHO DP12 cell; and wherein further the heterologous immunoglobulin is selected from the group consisting of an anti-AB